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EFFECT OF SALT STRESS ON ACID PHOSPHATASE ACTIVITY AND PHOSPHORUS CONTENT OF *Lycopersicon peruvianum* L. UNDER *IN VITRO* CULTURE

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ABSTRACT

In this study, the effect of different concentrations of NaCl (0, 60, 90, 120 and 150 mM NaCl) on phosphorus content and acid phosphatase (APase) activity in *Lycopersicon peruvianum* L were investigated. Results indicated that phosphorus content was decreased and Apase activity was increased at 150 mM NaCl. With increasing salinity levels phosphorus content of roots decreased in all NaCl concentrations, but the Apase activity increased significantly with increasing salt concentrations. Salt stress did not change the relative chlorophyll content of leaves. It can be concluded that salt stress may induce APase activity accompanied by a low level of P_i.

Key words: Tomato, Salt stress, Phosphorus, acid phosphatase

INTRODUCTION

Tomato, one of the important and widespread crops in the world, is sensitive to moderate levels of salt in the soil. Many studies have reported large variation among tomato genotypes in their response to salinity (Ben Ahamed et al., 2009). *Lycopersicon peruvianum* , a wild tomato species is an abundant source of valuable agricultural traits including resistance to fusarium root rot, leaf mold, tomato yellow leaf virus and root-knot nematode. In addition, *Lycopersicon peruvianum* is a source of genes for tolerance development of salt and high ascorbic acid levels in cultivated tomato (Doganlar and Tanksley, 1997). One of the important gene is *Mi* gene, which was transferred from *Lycopersicon peruvianum* into cultivated tomato confers resistance to root- knot nematodes in cultivated tomatoes in1940s (Ammiraju et al., 2003). *Mi* is genetically attached tightly to Acid phosphatase (APase) (Ho et al., 1992). Phosphorous (P_i) is an important macronutrient that constitutes vital molecules such as nucleic acid, phospholipids and sugar phosphates in all living organisms. It makes up about 0.2% of plant dry weight. Terrestrial plants generally meet their P_i requirement by the uptake of soil P_i in inorganic form. However, a considerable fraction of the soil P (50- 80%) exists as organic compounds which are unavailable to plants unless mineralization take place (Starnes et al., 2008). Efficient acquisition and utilization of phosphorus requires a ubiquitous class of enzymes known as phosphatases which function to hydrolyze P_i from orthophosphate monoesters and have been traditionally classified as being alkaline or acid phosphatases according to their optimal pH for catalysis above or below pH 7.0 (Duff et al., 1994). Acid phosphatases are known to act under salt stress by maintaining a certain level of P_i (Olmos and Hellin, 1997). It is considered that the phosphorus deficiency observed in the selected pea calli can serve as a signal for the induction of acid phosphatase (Lefebvre et al., 1990) and also has been reported an increased level of phosphatase activity accompanied by a decrease in a phosphorus under salt stress in some varieties of wheat calli (Olmos and Hellin, 1997). If there is a direct correlation between acid phosphatase activity and salt stress then it would be worthwhile to isolate acid phosphatase encoding gene and transform the plant with this gene and increase level of salt tolerance of plant by over expression of this gene. However, this study is aimed to investigate relation between the activity of acid phosphatase enzyme, phosphorus content and salt tolerance.

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MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon peruvianum*, line 825), were surface-sterilized with 20% sodium hypochlorite solution for 10 min and then rinsed with sterile distilled water. Seeds were then germinated in MS medium (Murashige and Skoog, 1962) containing concentrations of 0, 60, 90, 120 to 150mM NaCl. Plants were grown in a controlled culture room under artificial light (1500 lux, 16/8h light-dark photoperiod), at 25°C. After 4 weeks stem-leaf and root segments were cut and phosphorus content as well as acid phosphatase activity was measured.

To measure phosphorus content, segments of leaf-stem and root were obtained from salt treated and untreated plants. Samples were dried at 70°C for at least 72h. Dried samples were heated at 550-600°C for 7-8h and white ash was produced. P_i was extracted with diluted nitric acid with distilled water (1:2) and aliquots, were mixed with 15ml ammonium Vanadate reagent. (This reagent, reacts with P_i of solution and forms yellow complex). The absorption was determined using spectrophotometer at 405nm.

For measurement of acid phosphatase activity about 0.1g of fresh tissue from salt treated as well as untreated roots and stem-leaves were harvested. The fresh samples were then grounded in a cold mortar, and macerated in 5ml of 0.2 M sodium acetate-acetic acid buffer (pH 5.8). The extracts were then centrifuged at 12000 rpm for 10 min at 4°C. Supernatants then were used for APase activity determination. The activity of enzyme was assayed using p-nitro phenyl-phosphate as substrate (McLachlan et al., 1987). Data were statistically analyzed using ANOVA and means were compared according to Tukey test by Sigma Stat2 software and illustrated using Excel software.

Relative chlorophyll content was measured using colorimeter set (MINOLTA-SPAD-502 JEPAN). Relative chlorophyll content of 10 randomly selected leaves was measured. Mean data from 5 replications was used for statistical analysis.

RESULTS

Phosphorus content of aerial parts of salt treated plants is shown in Figure 1. Results showed that up to 120 mM NaCl there was no significant difference in phosphorus content between untreated and treated plants. However a significant decrease between, 150 mM NaCl and other salt treated plants was observed. In contrast, by increasing of salt concentration in the culture medium, phosphorus content decreased significantly in roots compared to untreated plants (Figure 2).

Acid phosphatase activity in stem-leaf parts of salt treated plants is shown in Figure 3. Increasing salt content of the culture medium at 60, 90 and 120 mM NaCl did not increase the enzyme activity. But the enzyme activity was increased dramatically at 150 mM NaCl compared to untreated plants. However, acid phosphatase activity in roots was basically different from stem-leaf. Salt treated roots showed significant increase in enzyme activity compared to the control plant. The maximum enzyme activity was obtained at 90 mM NaCl but at 120 and 150 mM enzyme activity was decreased significantly.

When salt concentration was increased in the medium relative chlorophyll content did not change

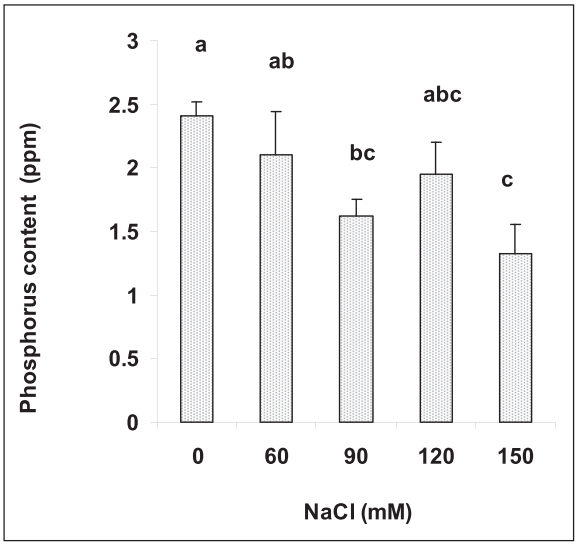

Figure 1. Phosphorus content in stem-leaf of *Lycopersicon peruvianum*. (Uncommon letters are significant P<0.05 based on Tukey test)

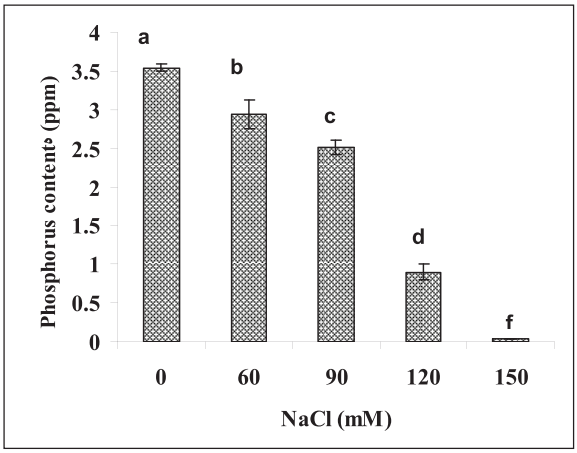

Figure 2. Phosphorus content in root of *Lycopersicon peruvianum*. (Uncommon letters are significant P<0.05 based on Tukey test)

up to 90 mM NaCl, but at 120 and 150 mM there was a significant decrease in chlorophyll content in salt streated at 0, 60 and 90 mM (Figure 5).

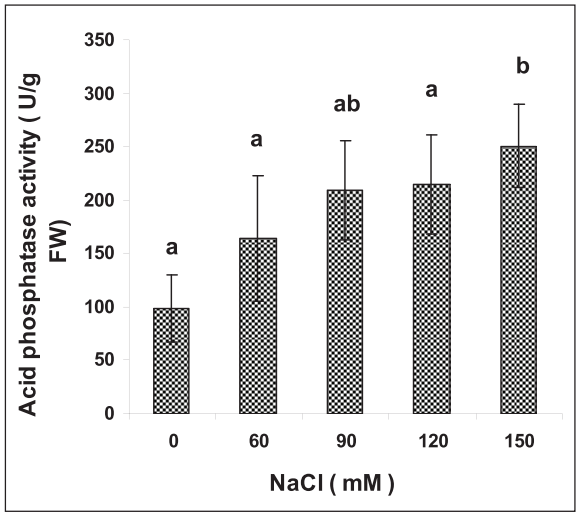

Figure 3. Effect of salt stress on acid phosphate activity in stem-leaf of *Lycopersicon peruvianum*. (Uncommon letters are significant P<0.05 based on Tukey test)

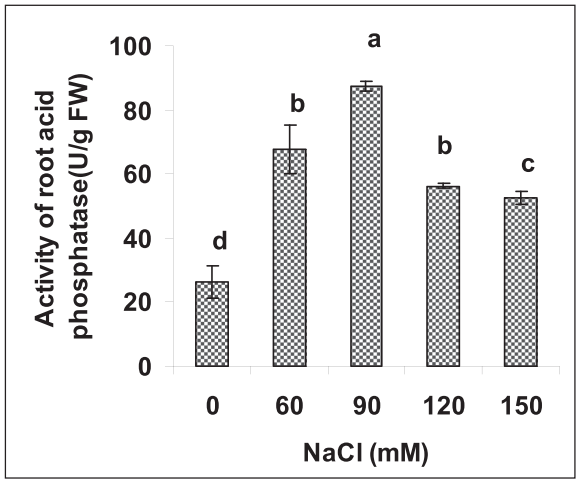

Figure 4. Effect of different concentrations of salt on acid phosphate activity in root of *Lycopersicon peruvianum*. (Uncommon letters are significant P<0.05 based on Tukey test)

DISCUSSION

Acid phosphatases in plants is a class of enzymes that display considerable heterogeneity with regard to their kinetics and functions (Duff et al., 1994). This complexity may contribute to conflicting reports in the literature regarding the relationship between APase and phosphorus deficiency (Yan et al., 2001). To understand the relationship between APase, salt stress and phosphorus stress, we carried out the present study. In this report, with increasing salt concentration to 120 mM NaCl, phosphorus content of stem-leaf parts of *Lycopersicon peruvianum* did not show any considerable change, but at 150mM NaCl significant decrease was observed. The relation between acid phosphatase activity and salt stress has recently been reported in algae (Chakraborty, et al., 2010). These results are in general agreement with those reported about phosphorus content of wheat calli under salt stress (Olmos and Hellin, 1997). Lack of significant decrease in phosphorus content under salt stress at 120mM in aerial parts of plant can be related to genetic tolerance of the plant (Trivedi et al., 1991). Our results suggest most likely that increase in APase activity correlates with a low level of P_i in this plant. In contrast, significant decrease occurred in phosphorus content of plant root under salt stress in all salt treated plants (Figure 2). This finding suggested that root is more prone to phosphorus deficiency compared to aerial organs. It should be considered that roots are exposed directly to salt stress in culture medium while, aerial organs are affected later indirectly. It is obvious that changes of phosphorus content are more noticeable in root than aerial organs.

Decrease of P_i might be a consequence of severe salt stress. In stem leaf, high level of acid phosphatase activity (Figure 3) was observed by increasing salt concentration. At 150 mM NaCl highest level of enzyme activity was observed, however the difference between 150mM and other salt treatments was not significant and it might be due to transport of P_i from root to shoot and leaf. When we looked at the activity of enzymes in roots, increasing level of APase activity was observed by increasing level of salt concentrations. One speculation is the direct and severe exposure of roots to salt stress. By increasing salt concentration the

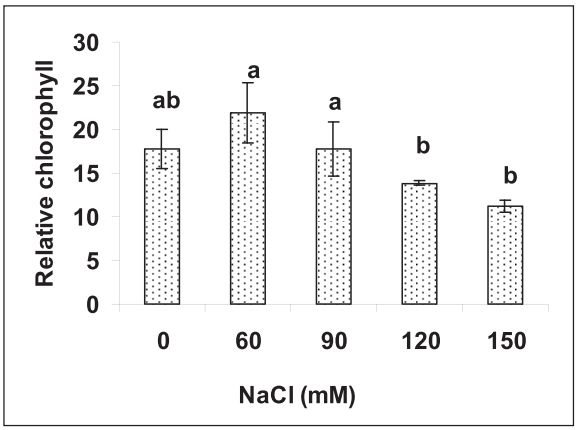

Figure 5. Effect of salt stress on relative chlorophyll in leaves of *Lycopersicon peruvianum*. (Uncommon letters are significant P<0.05 based on Tukey test)

activity of APase in roots increased. The highest level of APase in roots was observed at 90 mM NaCl while at 120 and 150 mM activity of enzyme was significantly lower than 90 mM. However, it should be noted that the activity of enzyme at 120 and 150 mM NaCl still is much higher than untreated plants (control). Lower enzyme activity at 120 and 150 mM might be due to high concentration of salt in the culture medium, low level of P_i in root cells and perhaps disruption in enzyme activity. The increased level of APase activity correlating with a low level of P_i in numerous plant species including tomato leaves, wheat leaves and roots, subterranean clover, karri and cultured tobacco cells have been reported (Szabo-Nagy et al., 1992). It can be concluded that there is a reverse relation between phosphorus content and acid phosphatase activity in *Lycopersicon peruvianum*. Phosphorus deficiency can make a signal for induction of acid phosphatase activity. Acid phosphatases are known to act under salt stress by maintaining a certain level of P_i which can be co-transported with H^+ along a gradient of proton motive force (Olmos and Hellin, 1997).

Salt stress leads to chlorophyll changes in leaf. Relative chlorophyll content of leaves was unchanged up to 90 mM NaCl while a significant decrease in chlorophyll content was observed at 120 and 150 mM salt. This is expected as salt stress normally affects photosynthesis machinery and decreases chlorophyll content, producing reactive oxygen species (ROS). (Agastian et al., 2000, Wenxue et al., 2003, Netondo et al., 2004).

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